

The Interaction of Atovaquone with the *P. carinii* Cytochrome bc₁ Complex

STEVEN R. MESHNICK,^{a*} EDWARD A. BERRY,^b JURGEN NETT,^c POWEL KAZANJIAN,^a BERNARD TRUMPOWER^c

^aUniversity of Michigan, ^bLawrence Berkeley National Laboratory, ^cDartmouth Medical School

Atovaquone is an important second-line therapeutic and prophylactic agent for *Pneumocystis carinii* pneumonia although it was originally developed as an antimalarial [11]. For malaria, atovaquone is now only used in combination with proguanil, another antimalarial. This combination (Malarone[®]) is used because atovaquone monotherapy leads to resistance [9].

Atovaquone is structurally similar to ubiquinolone (CoQ) (Fig. 1). UHDBT and stigmatellin (Fig. 1) are other CoQ analogs that have been well studied in experimental systems [2, 3]. Both UHDBT and stigmatellin are known to inhibit electron transport by binding to the Q₀ site of the cytochrome bc₁ complex. Atovaquone probably acts at the same site, since it has also been shown to inhibit electron transport in both malaria and *P. carinii* [4, 6]. This mechanism could also explain the rapid emergence of resistance to monotherapy; mutations could arise easily in cytochrome b, since it is encoded in the mitochondrial genome where the spontaneous mutation rate is 10x higher than in nucleus [1].

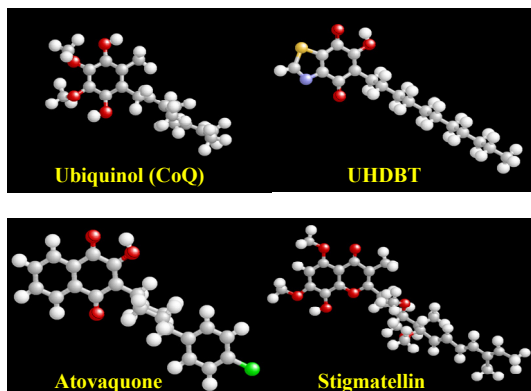


Fig.1 Structures of ubiquinolone and its analogs.

In experimental systems, resistance to UHDBT and stigmatellin has been shown to arise by mutations leading to changes in amino acids in cytochrome b that line the Q₀ site [2, 3]. In 1998, we found mutations in the *P. carinii* cytochrome b Q₀ site in isolates from patients exposed to atovaquone [14]. Subsequently, mutations in the cytochrome b Q₀ site were found to be associated with atovaquone resistance in laboratory strains of malaria and toxoplasmosis [8, 10, 12, 13]. We recently demonstrated a significant association between mutations in the *P. carinii* cytochrome b Q₀ site and exposure to atovaquone in a retrospective cohort study [7]. The sites of mutations in this and the previous study are shown in Figure 2.

Our goal now was to determine the location of these mutated amino acids in relation to the probable binding site of atovaquone. Since the *P. carinii* cytochrome bc₁ complex has not been crystallized, we used the yeast and

chicken bc₁ complexes, whose 3-dimensional structures are known (PDB ID codes 2BCC and 1EZV) as models [5, 15].

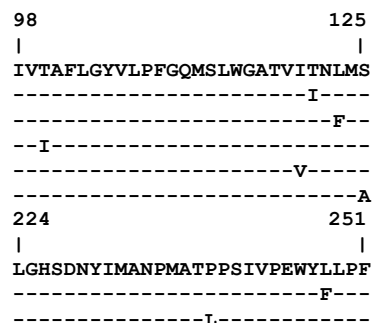


Fig. 2. Mutations found in the *P. carinii* cytochrome b Q₀ site (see refs [7] and [14]).

We first used a molecular mechanics energy minimization program, MacSpartan Pro, to obtain the lowest energy conformation of atovaquone (Fig. 3).

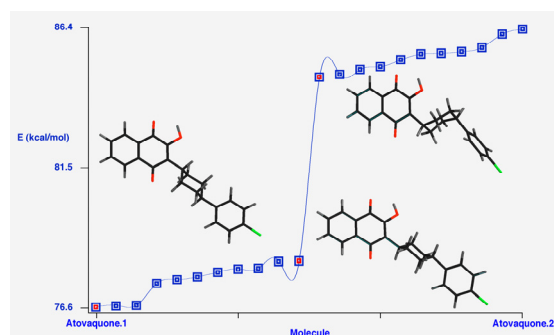


Fig. 3. Conformational energy profile for atovaquone.

Model-building based on the stigmatellin complexes suggests that atovaquone could bind to the bc₁ complex in either of two similar orientations. The correct binding mode will probably be unknown until co-crystals are obtained, however both orientations fit in the same cavity (data not shown). The interactions between atovaquone and the cytochrome bc₁ complex are shown for the yeast (Fig. 4) and chicken (Fig. 5) complexes. Amino acids which are homologous to the mutated amino acids are shown with bonds that are yellow (for *P. carinii*) and green (for *Toxoplasma* and *Plasmodium*). Amino acids in the yeast complex which are homologous to 5 of the *P. carinii* mutations (I147, T148, L150, S152, L275) in close proximity to atovaquone, while 2 (T127 and P266) are not. These latter mutations must affect atovaquone binding indirectly. For comparison, amino acids which are mutated in toxoplasma (M139 and I269) and plasmodium (I269, F278, Y279, L282, and R283) are also shown as is the iron-sulfa protein (purple) and the

* Corresponding author: Steven R. Meshnick – Telephone: 734 647-2406; Fax: 734-764-3192; Email: meshnick@umich.edu

active site histidine (H181). This pattern is the same for the chicken structure (Fig. 5).

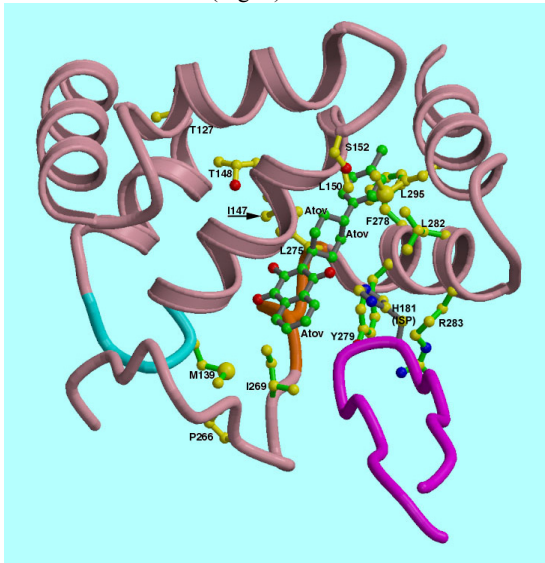


Fig.4. Interaction of atovaquone with yeast cytochrome bc_1 complex.

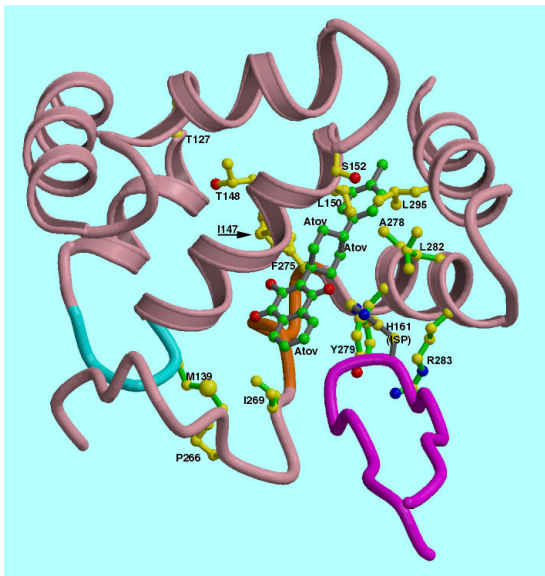


Fig.5. Interaction of atovaquone with avian cytochrome bc_1 complex.

We have also begun studying the effects of atovaquone on the yeast and bovine cytochrome bc_1 complexes. As is shown in Figure 6, atovaquone is a competitive inhibitor. At a concentration of $0.1 \mu\text{M}$, which inhibits the yeast enzyme approximately 50%, atovaquone increases the apparent K_m for ubiquinol and has no effect on V_{max} . In titration experiments, atovaquone causes 50% inhibition of the yeast enzyme at 90 nM and of the bovine enzyme at 170 nM . Thus, atovaquone appears to be selective for the yeast enzyme compared to the bovine enzyme. Of interest, yeast, *P. carinii*, *Toxoplasma* and *Plasmodium* ("sensitive") cytochrome b 's have aromatic residues at position 278,

while the bovine, chicken and human cytochrome b 's do not. In *Plasmodium*, resistance develops when this aromatic amino acid is changed to isoleucine. These data suggest that a pi-pi interaction between this residue and atovaquone might promote binding.

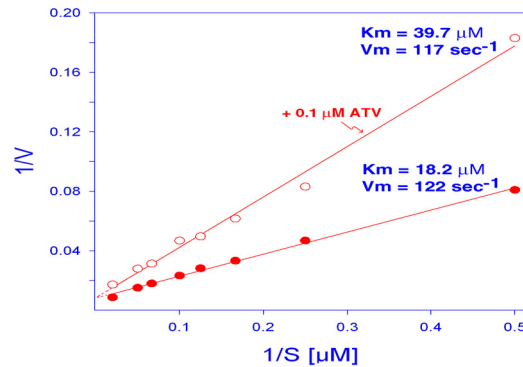


Fig. 6. Lineweaver-Burke plot showing competitive inhibition of the yeast cytochrome bc_1 complex by atovaquone (ATV)

Further studies on the interaction between atovaquone and cytochrome bc_1 may lead to a better understanding of the mechanism of resistance and aid in the development of new chemotherapeutic agents.

Acknowledgements

We would like to thank Wendy Armstrong, Paul A. Hossler, Ben Beard, Laurence Huang, Jane Carter, Jeffrey Duchin, Lawrence Crane, William Burman, James Richardson for technical help and access to patient samples. This work was supported by NIH grants RO1 AI 46966, GM 20379, and R01 DK 44842.

References

1. Albers, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J.D. Watson. 1989 Molecular biology of the cell, 2nd ed., Garland Publishing, Inc., New York. 1219.
2. Berry, E.A., M. Guergova-Kuras, L.S. Huang, and A.R. Crofts. 2000. Structure and function of cytochrome bc complexes. *Annu Rev Biochem* **69**:1005-75.
3. Esposti, M.D., S. De Vries, M. Crimi, A. Ghelli, T. Patarnello, and A. Meyer. 1993. Mitochondrial cytochrome b : evolution and structure of the protein. *Biochim Biophys Acta* **1143**:243-71.
4. Fry, M. and M. Pudney. 1992. Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(4'-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). *Biochem Pharmacol* **43**:1545-53.
5. Hunte, C., J. Koepke, C. Lange, T. Rossmann, and H. Michel. 2000. Structure at 2.3 \AA resolution of the cytochrome $bc(1)$ complex from the yeast *saccharomyces cerevisiae* co-crystallized with an antibody Fv fragment. *Structure Fold Des* **8**:669-84.
6. Ittarat, I., W. Asawamahakda, M.S. Bartlett, J.W. Smith, and S.R. Meshnick. 1995. Effects of

- atovaquone and other inhibitors on *Pneumocystis carinii* dihydroorotate dehydrogenase. *Antimicrob Agents Chemother* **39**:325-8.
7. Kazanjian, P., W. Armstrong, P.A. Hossler, L. Huang, C.B. Beard, J. Carter, L. Crane, J. Duchin, W. Burman, J. Richardson, and S.R. Meshnick. 2001. *Pneumocystis carinii* cytochrome b mutations are associated with atovaquone exposure in patients with AIDS. *J Infect Dis* **183**:819-22.
 8. Korsinczky, M., N. Chen, B. Kotecka, A. Saul, K. Rieckmann, and Q. Cheng. 2000. Mutations in *Plasmodium falciparum* cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. *Antimicrob Agents Chemother* **44**:2100-8.
 9. Looareesuwan, S., C. Viravan, H.K. Webster, D.E. Kyle, D.B. Hutchinson, and C.J. Canfield. 1996. Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. *Am J Trop Med Hyg* **54**:62-6.
 10. McFadden, D.C., S. Tomavo, E.A. Berry, and J.C. Boothroyd. 2000. Characterization of cytochrome b from *Toxoplasma gondii* and Q(o) domain mutations as a mechanism of atovaquone-resistance. *Mol Biochem Parasitol* **108**:1-12.
 11. Spencer, C.M. and K.L. Goa. 1995. Atovaquone. A review of its pharmacological properties and therapeutic efficacy in opportunistic infections. *Drugs* **50**:176-96.
 12. Srivastava, I.K., J.M. Morrissey, E. Darrouzet, F. Daldal, and A.B. Vaidya. 1999. Resistance mutations reveal the atovaquone-binding domain of cytochrome b in malaria parasites. *Mol Microbiol* **33**:704-11.
 13. Syafruddin, D., J.E. Siregar, and S. Marzuki. 1999. Mutations in the cytochrome b gene of *Plasmodium berghei* conferring resistance to atovaquone. *Mol Biochem Parasitol* **104**:185-94.
 14. Walker, D.J., A.E. Wakefield, M.N. Dohn, R.F. Miller, R.P. Baughman, P.A. Hossler, M.S. Bartlett, J.W. Smith, P. Kazanjian, and S.R. Meshnick. 1998. Sequence polymorphisms in the *Pneumocystis carinii* cytochrome b gene and their association with atovaquone prophylaxis failure. *J Infect Dis* **178**:1767-75.
 15. Zhang, Z., L. Huang, V.M. Shulmeister, Y.I. Chi, K.K. Kim, L.W. Hung, A.R. Crofts, E.A. Berry, and S.H. Kim. 1998. Electron transfer by domain movement in cytochrome bc1. *Nature* **392**:677-84.